

## Some silkweeds (Zygnemataceae, Zygnemales, Charophyceae) from the Upper Murrumbidgee River catchment

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### Abstract

Twenty two species of Zygnemataceae, two species of *Mougeotia*, *Sirogonium sticticum*, fourteen species of *Spirogyra* and five of *Zygnema* were encountered in recent collections of macroalgae from the Upper Murrumbidgee River catchment in the Australian Capital Territory and Southern Tablelands of New South Wales, Australia. The majority of the taxa collected were newly reported for Australia. The occurrence and distribution of silkweeds in both flowing water and wetlands in the catchment is similar to that described for other semi-arid parts of the world, notably Spain, south-western United States and the coastal areas of the Netherlands.

### Introduction

The silkweeds (Zygnemataceae) are commonly encountered freshwater algae and so have a high potential as indicator species for water quality and aquatic habitat status. There is little information about the ecological requirements of individual species, both in Australia and elsewhere. Just as importantly, these algae are most frequently encountered in the vegetative state in the field. Induction of reproduction is involved, and often has a low success rate (Simons et al., 1984). Without reproductive material classification is limited to little more than genus, and sometimes only provisionally. This report is a first step in addressing these matters for the Upper Murrumbidgee River catchment.

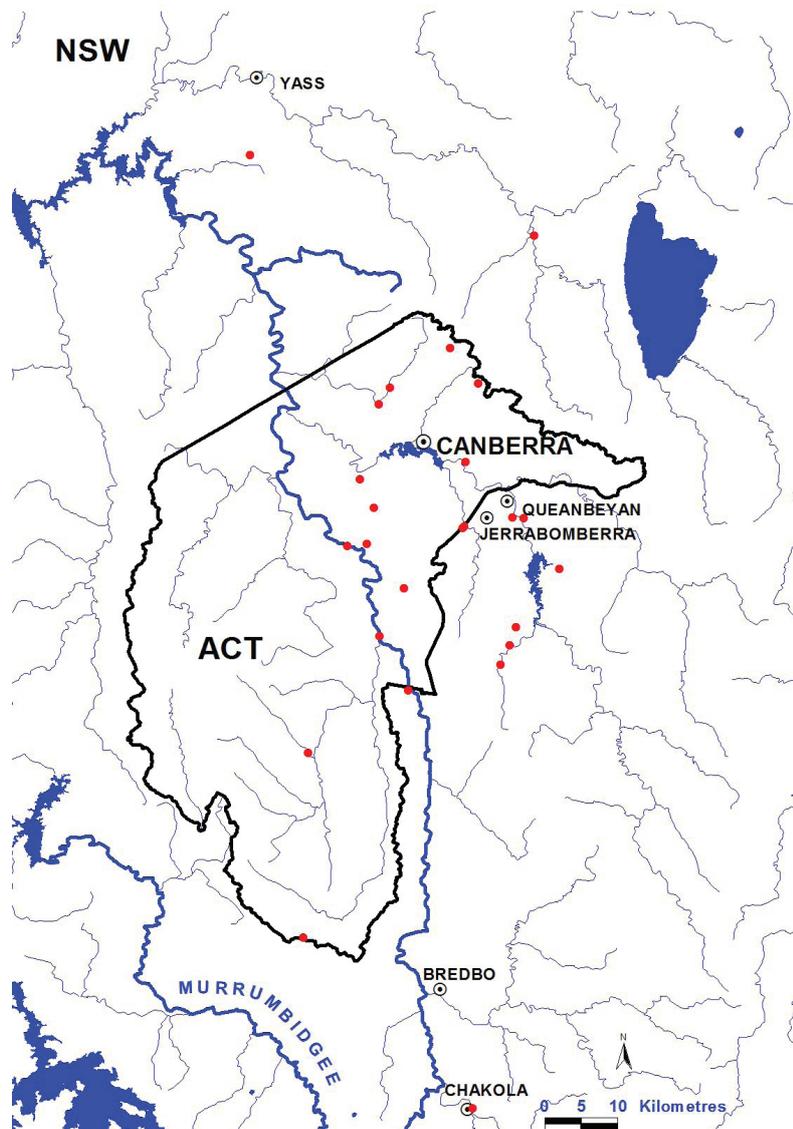
Lists of species or descriptions of regional freshwater algal floras for river catchments in New South Wales, and in Australia, are occasionally found in the technical reports of government departments and catchment authorities. Occasionally papers are published which include descriptions and bio-geographical information (Townsend et al 2008) but generally the baseline information is unavailable, being held in departmental records (Skinner 2008). It is rare to find published papers with detailed and floristic content (May and Powell 1986, Entwisle 1989a, b).

The Algae of Australia treatment (Lewis and Entwisle 2007) presented those taxa in the Zygnemataceae that could be verified from specimens, and discussed the status of other records. It forms a baseline for this study, which presents thirteen new species records, while reinstating one doubtful and two rejected species for the continent.

## Study Area

The Upper Murrumbidgee River catchment includes the river and its tributaries above Burrinjuck Dam in New South Wales (Fig. 1). The catchment includes several important rivers that drain most of the southern and western side of the Southern Tablelands. As the Murrumbidgee River system is the third most important contributor to the Murray–Darling River Basin, it is important to have its freshwater algal biodiversity documented.

The Murrumbidgee River rises in the Australian Alps, leaves Tantangara Reservoir and gathers in water from Cooma Creek, the Numeralla and Bredbo Rivers and enters the Australian Capital Territory (ACT) at Angle Bend. The Naas-Gudgenby system, including Grassy Creek, joins the Murrumbidgee at Tharwa village in the ACT. Tuggeranong Creek joins the Murrumbidgee near Pine Island, the Cotter (and Paddys) River upstream of Casuarina Sands and the Molonglo River opposite Woodstock Reserve in the ACT. The Murrumbidgee River reaches the Burrinjuck reservoir below Taemas Bridge on the Wee Jasper Road in New South Wales. The Yass River joins the Burrinjuck reservoir to form its eastern arm. The Cotter River is a rare example of a river in a protected catchment. The Queanbeyan River has much grazing country in the upper catchment, and meets the Molonglo River after passing through Queanbeyan, a city of 38,000 people. The Molonglo River is shallow and rain dependant, and used for agriculture and forestry, and mining in the recent past. It rises above Captains Flat, meanders across the Hoskinstown Plain and through the Molonglo Gorge joining with the Queanbeyan and Jerrabomberra Creek to form Lake Burley Griffin in the centre of Canberra. The Yass River valley has been an area of intense agriculture for almost 200 years.



**Fig.1.** Map of the Southern Tablelands of New South Wales and the Australian Capital Territory; position of collection sites marked as red dots, rivers and reservoirs in blue.

## Methods

For some time it has been demonstrated that the circumscription of species in the Zygnemales on morphological characters alone is at best imperfect and at worst biologically unsound. Hoshaw and McCourt (1988) reviewed the many publications on Zygnemataceae since Godward (1966). Among other comments, they warned:

‘The existence of species complexes argues against the further proliferation of new species descriptions based on minor morphological variations.’ (Hoshaw and McCourt 1988, p. 540)

However, morphologically distinct forms may help in field determinations, and inclusion of these as well documented biological units may enhance our understanding of the ecological significance of the appearance (or absence) of silkweeds in waterways. However, there is often little available genetic, ecological and physiological data to support a more holistic species concept.

In this paper the genera will not be separated into Sections, except in *Spirogyra* where informal groupings based on chloroplast number and end-wall form will be used. General descriptors indicating which morphological characters are common to groups of taxa, in the manner of Gauthier-Lièvre (1965) is provided.

Hainz et al. (2009) present a well-defined set of thirteen filament groupings, which they called morphotypes, for *Spirogyra* from central Europe based on cell width and number of chloroplasts that could be associated with environmental parameters. The environmental data collected in this study is insufficient to replicate that work. The Lewis and Entwisle (2007) separation of vegetative groups in *Spirogyra* is simplified here. The separation on the basis of end-wall form and number of chloroplasts is retained, but the width of the filaments is no longer used, giving four *Spirogyra* vegetative groups.

**Table 1. Vegetative groups in *Spirogyra* used here.**

Vegetative Group	End-wall form	Number of Chloroplast ribbons
<i>Spirogyra</i> A	Plane	Single, rarely 2
<i>Spirogyra</i> B	Plane	2–5, generally 4
<i>Spirogyra</i> C	Plane	6 or more
<i>Spirogyra</i> S	Replicated	1–2

The circumscription of species descriptions presented in this paper is based on field collections. Where there is information to indicate a broader species concept it will be included. As a minimum each description for a taxon requires the:–

- range of width of the vegetative cells;
- range in number of the chloroplast ribbons;
- general shape of the zygospore;
- colour of the mature zygospore; and
- ornamentation of the mesospore (Hoshaw *et al.*, 1987, McCourt and Hoshaw 1990).

While some samples were collected as part of vegetation surveys of the rivers of the region, many collections were opportunistic. Much of the material is preserved as semi-permanent slides with material mounted in Karo, and some are accompanied by small collections preserved in 70% ethanol. Specimens were examined with a Leitz Labalux research microscope and drawing arm. Collections are held in the National Herbarium of New South Wales (NSW).

Water quality data (see Appendix 1) are taken from the Upper Murrumbidgee Waterwatch database for the years 2007–2013 (<http://www.act.waterwatch.org.au/>).

Appendices 2 and 3 present the data upon which the biogeographical comments are based.

## Results

### Part 1: Species descriptions:

***Mougeotia*** C.Agardh, *Systema algarum* 83 (1824), *nom. cons.*

Type species: *M. genuflexa* (Roth) C.Agardh

*Zygospore formed in conjugation tube, with spurs into each end of the gametangial cells.*

***Mougeotia*** sp. aff. ***quadrangulata*** Hassall *Annals and Magazine of Natural History*, 12:434 (1843). Lewis and Entwisle *Zygnemaceae* : 114, fig. 33A (2007).

Type: Kent, England (BM n.v.)

Vegetative cells cylindrical, 11–12 µm diam., length/diameter (L/D) 5–10+, with paired saucer end-walls, and a broad ribbon chloroplast with 2–6 pyrenoids in a line.

Conjugation scalariform, with zygote forming in the disintegrating conjugation tube and the cell ends, quadrate and narrowly box-like with short spurs in each cell remnant, 27–39 µm long, 24–27 µm diameter, 18–25 µm; exospores clear and smooth, mesospore dull buff, smooth or with tiny spots. **Fig. 2a–c.**

**Distribution:** *Mougeotia quadrangulata* is found across Europe, North and South America, Africa generally and China. It has been previously reported in Australia in the Northern Territory (Lewis and Entwisle, 2007).

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: Burra Creek, 3 km downstream of the gauging station below Burra Road bridge, side pool as part of metaphyton, 15 May 2012 (S. Skinner 972.1)

This site generally has pH 8.0–8.9, Electrical Conductivity 430–600 µS.cm<sup>-1</sup>, and turbidity of <10 NTU. Flow is generally low, with pulses following rain storms.

This taxon keys to *M. quadrangulata* in Transeau (1951) and Kadłubowska (1984), but does not fit the descriptions very well. It has only 4–6 pyrenoids in the chloroplast in vegetative cells, has rounded, block-like spurs on the corners of the zygospores, and the punctae are well under 1µm across. As well, the arms of the gametangia are very short. In Randhawa (1959) it keys to *M. quadrata* Randhawa. Kolkwitz and Krieger (1941), and Kadłubowska (1984) place Randhawa's (1938) *M. quadrata* in *M. paludosa* G.S.West, but the present specimen has similarities with both *M. paludosa* and *M. quadrangulata*. The Burra Creek specimen agrees well with the dimensions in Randhawa (1959) for *M. quadrata* but the spurs on the corners of the zygospores are more pronounced. As *M. quadrangulata* is the oldest described taxon, and further work may reveal a species complex, it appears to be the most suitable name for affinity.

*Zygospore formed wholly within the conjugation tube*

***Mougeotia lamellosa*** Jao *American Journal of Botany* 23: 60, fig. 36–37 (1936). Jao 1988, *Flora Algarum Sinicum aquae dulcis*, 1: 42, Pl. XL, figs 1–4. Kadłubowska 1984, *Zygnemales*: 68, fig 75. Randhawa 1959, *Zygnemaceae*: 137, fig 38. Transeau 1951, *Zygnemataceae*: 97, Pl. XV, figs 18–19.

Type: rice farm, Kiangpei, China, Jan 1933 MICH, No S404:M1) n.v.

Vegetative cells cylindrical, 28–33 µm diam., L/D 7–10+, paired saucer end-walls, and a broad ribbon chloroplast with 8–12 prominent pyrenoids more or less in two rows.

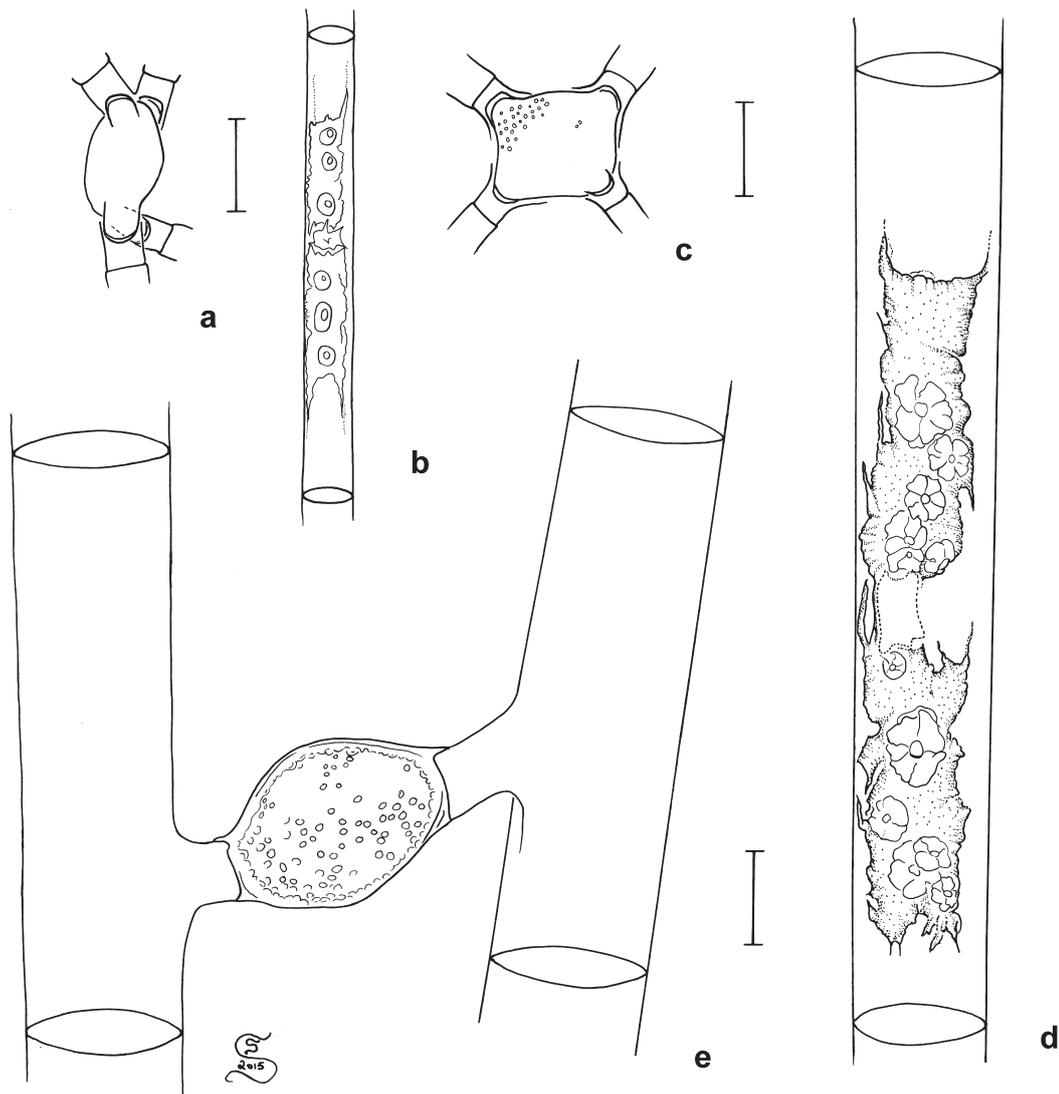
Conjugation scalariform; zygospore formed wholly within the persistent conjugation tube, lozenge shaped with short ends perpendicular to filaments in the retained ends of the conjugation tube, (38–) 43–50 µm long, 29–33 (–36) µm short axis; exospores clear, thick and lamellate in the conjugation tube, mesospore golden brown, very finely scrobiculate all over. **Fig. 2d, e.**

**Distribution:** south western China, France; reported in Australia for the first time.

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: Doyle Reserve, Barracks Flat, Queanbeyan, drain and ephemeral swamp, as rafts among sedges in water less than 10 cm deep, 23 Oct 2013 (S. Skinner 1012.1).

No water quality data.

Although the dimensions are slightly larger, the Doyle Reserve material is a close fit for the description in Jao (1936) and Jao (1988). The difference in sculpturing (dimples rather than warts) is distinctive; however, while *M. robusta* (de Bary) Wittrock and *M. subellipsoidea* Islam also have ellipsoid zygospores with dimples, neither is reported to have a thickened, lamellose exospore. There may be a species complex around *M. robusta*.



**Fig. 2.** a, b, c *Mougeotia* sp. aff. *M. quadrangulata*, a, c zygospore, side and top views, b, vegetative cell (Burra Creek, NSW, S. Skinner 972); d, e *M. lamellosa*, d, vegetative cell, e, mature zygospore (Doyle Reserve, Barracks Flat, Queanbeyan, NSW, S. Skinner1012.1) All scales 20  $\mu\text{m}$ .

***Sirogonium*** Kützing, *Phycologia Generalis* 278 (1843)

Type: *S. sticticum* (Sm.) Kützing

***Sirogonium sticticum*** (Sm.) Kützing, *Phycologia Generalis* 278 (1843)

*Conferva stictica* Sm. *English Botany*, Plate 2463, fig. A (1813)

*Choaspis stictica* Kuntze, *Revisio generum plantarum*, 2: 887 (1891)

Type: England, probably not designated.

Vegetative cells, broad and cylindrical, 48–52(–62)  $\mu\text{m}$  diam., L/D 3.5–8, end walls plane; 3–5(or 6) chloroplast ribbons, straight or slightly sigmoid, numerous pyrenoids.

Conjugation by adhesion; donor cell a short (L/D 1–1.5, 48–50  $\mu\text{m}$  diam.) cell in (or sometimes terminal to) donor filament, adheres to the lateral wall of the receptor cell; receptor cell inflated towards the donor cell, tending to barrel-shaped, 125–170  $\mu\text{m}$  long, 110–125  $\mu\text{m}$  diam.). Zygote formed wholly within the receptor, irregular ovoid, variable, 109–131  $\mu\text{m}$  long, (76–)90–109  $\mu\text{m}$  diam.; exospore clear, thin; mesospore thick, smooth or with very fine connective wrinkles, red-brown.

**Distribution:** Cosmopolitan; in Australia South Australia, Victoria and New South Wales (as *S. floridianum* Transeau)

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: Namadgi National Park: Orroral River, causeway, Orroral River Campground, 17 Nov 2007 (*S. Skinner* 909) Beltana St, Pialligo, soil under nursery benches, 22 Sep 2007 (*S. Skinner* 889) and 27 Oct 2007 (*S. Skinner* 897 & *O. Petkovic*) Orroral River pH 7.0–7.5, 50–95  $\mu\text{S}\cdot\text{cm}^{-1}$ , <10–15 NTU; Pialligo. no water quality data available.

Borge (1913), citing as *Spirogyra stictica* (Engl. Bot.) Wille, provided a wide definition for *S. sticticum* to encompass three of Kützing's taxa and *Choaspis stictica* Kuntze and allowed for 2–6 chloroplasts and 38–62  $\mu\text{m}$  diameter cells. Hoshaw (1980) retained the separation of *S. sticticum* and *S. floridianum* at 56  $\mu\text{m}$  in Key B, having reduced the number of taxa by some amalgamations, including *S. megasporum* (Jao) Transeau within *S. sticticum*.

*S. Skinner* 889 and *S. Skinner* 897 clearly fit *S. sticticum*. There are 3 or 4 almost straight chloroplasts and the vegetative cell width is 48–51  $\mu\text{m}$ . A few of the donor cells display the rudiments of a wide donor conjugation tube.

*S. Skinner* 909 has 5 or 6 slightly sigmoid chloroplasts and the cell diameter range is 51–59  $\mu\text{m}$ . While these characters are those for *S. floridianum* Transeau, the separation of taxa on cell width alone is not well supported in the Zygnematales. However Lewis and Entwisle (1998) reviewed the older records for these two taxa, and note that the cell diameter range given for the collections from the New England tableland (60–65  $\mu\text{m}$  diameter for vegetative cells, 5 chloroplasts; Skinner 1980) suit *S. floridianum*.

Stancheva et al. (2013) have shown that *Sirogonium* may be genetically part of one of two groups of *Spirogyra* with numerous chloroplasts per cell. For the present the genus is retained here.

***Spirogyra*** Link in C.G.D Nees von Esenbeck, *Horae Physicae Berolinensis* 5 (1820)

T: *S. porticalis* (O.F.Müll.) Cleve

Vegetative cells usually short (Length/Diameter ratio (L/D) <1.5), end-walls plane, with numerous (6 or more) closely packed chloroplast ribbons ***Spirogyra* C**

***Spirogyra maxima*** (Hassall) Wittrock in Wittrock & Norsdtedt, *Algae aquae dulcis exsiccatae* Nr. 460 (1882);

var. ***minor*** Moebius in *Abhandlungen der Senckenbergischen naturforschenden Gesellschaft. Frankfurt au Maine*, Bd18:334(1894); Bailey in *Botany Bulletin No. XI, Department of Agriculture, Brisbane*: 35, plate IX, figs 2–4 (1895)

*Spirogyra moebii* Transeau in Transeau et al. *Transactions of the American Microscopical Society* 53: 225 (1934).

Type: Dalby, Queensland, May 1893 (TL Bancroft) (herbarium Frankfurt, lost in WWII)

Vegetative cells shortly cylindrical, 66–83  $\mu\text{m}$  diam., L/D 1–3(–4), end-walls plane; 8–10 chloroplast ribbons,  $\frac{1}{2}$ –1 turn, with numerous small pyrenoids.

Conjugation scalariform or incomplete; donor and receptor cells similar to vegetative cells; conjugation tube two funnels; zygospore forms in receptor. Zygospore domed, round to slightly ovoid lenticular, 59–71  $\mu\text{m}$  long, 66.5–78  $\mu\text{m}$  diam., exospore clear, thick; mesospore with smooth rim and irregularly reticulate and pitted surface, red-brown. **Fig. 3b, c.**

**Distribution:** Cosmopolitan, in Australia reported from Queensland (see Lewis and Entwisle 1998). Collections which may fit this species have been made in other parts of the Murray–Darling basin, but await further study.

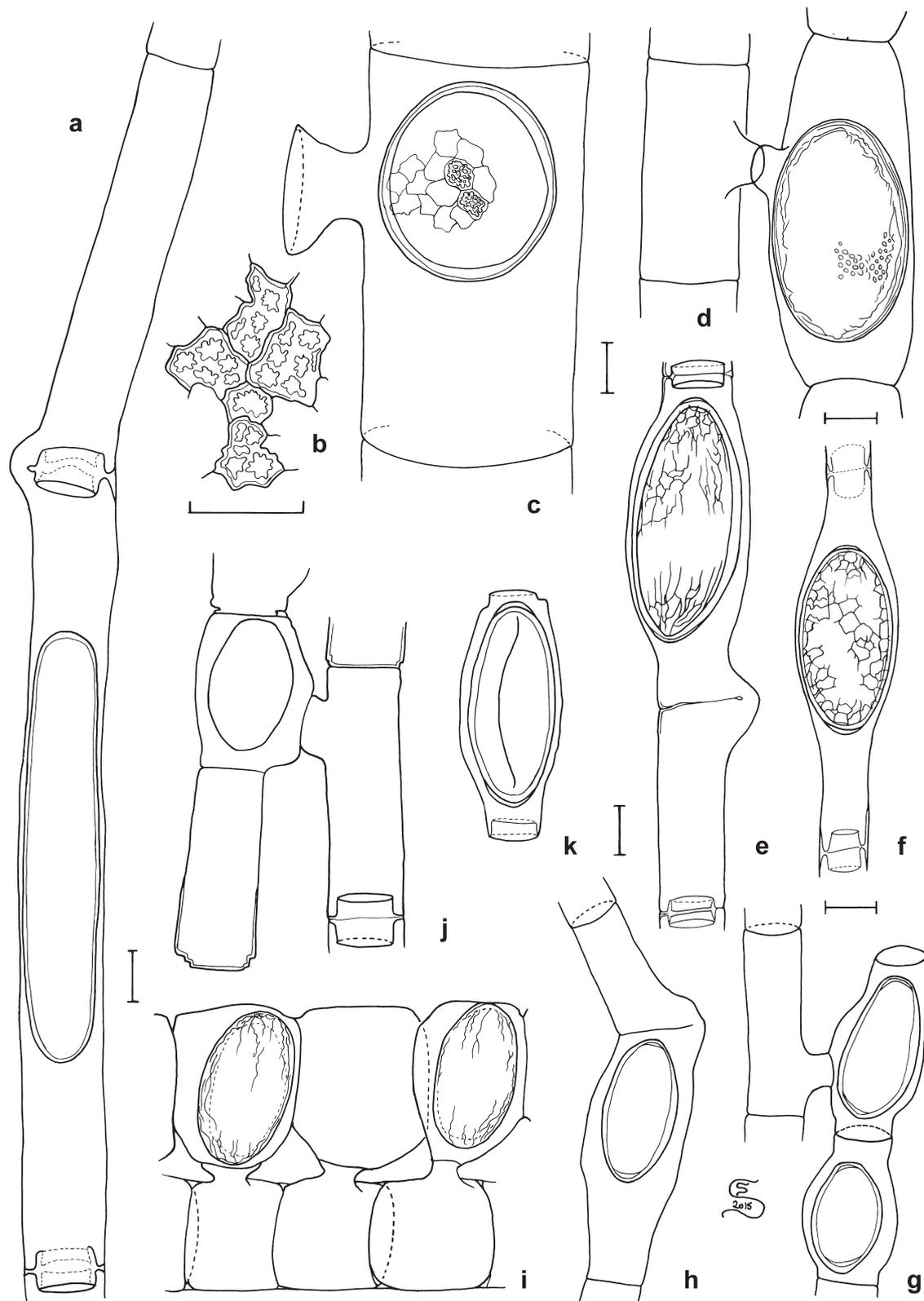
**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tableland: mouth of Freshford Creek, Red Rocks Gorge, Murrumbidgee River Corridor, 29 Feb 2008 (*S. Skinner* 934 & *L. Johnston*); waterfall, creek in John Knight Memorial Park, Lake Ginninderra, 16 Oct 2007 (*S. Skinner* 896) [this constructed wetland is filled from the East Arm of Lake Ginninderra]

Freshford Creek pH 7.8–8.7, EC 75–160  $\mu\text{S}\cdot\text{cm}^{-1}$ , Turbidity <10–20 NTU; Lake Ginninderra pH 7.1–8.3, EC 140–320  $\mu\text{S}\cdot\text{cm}^{-1}$ , Turbidity <10–56 NTU.

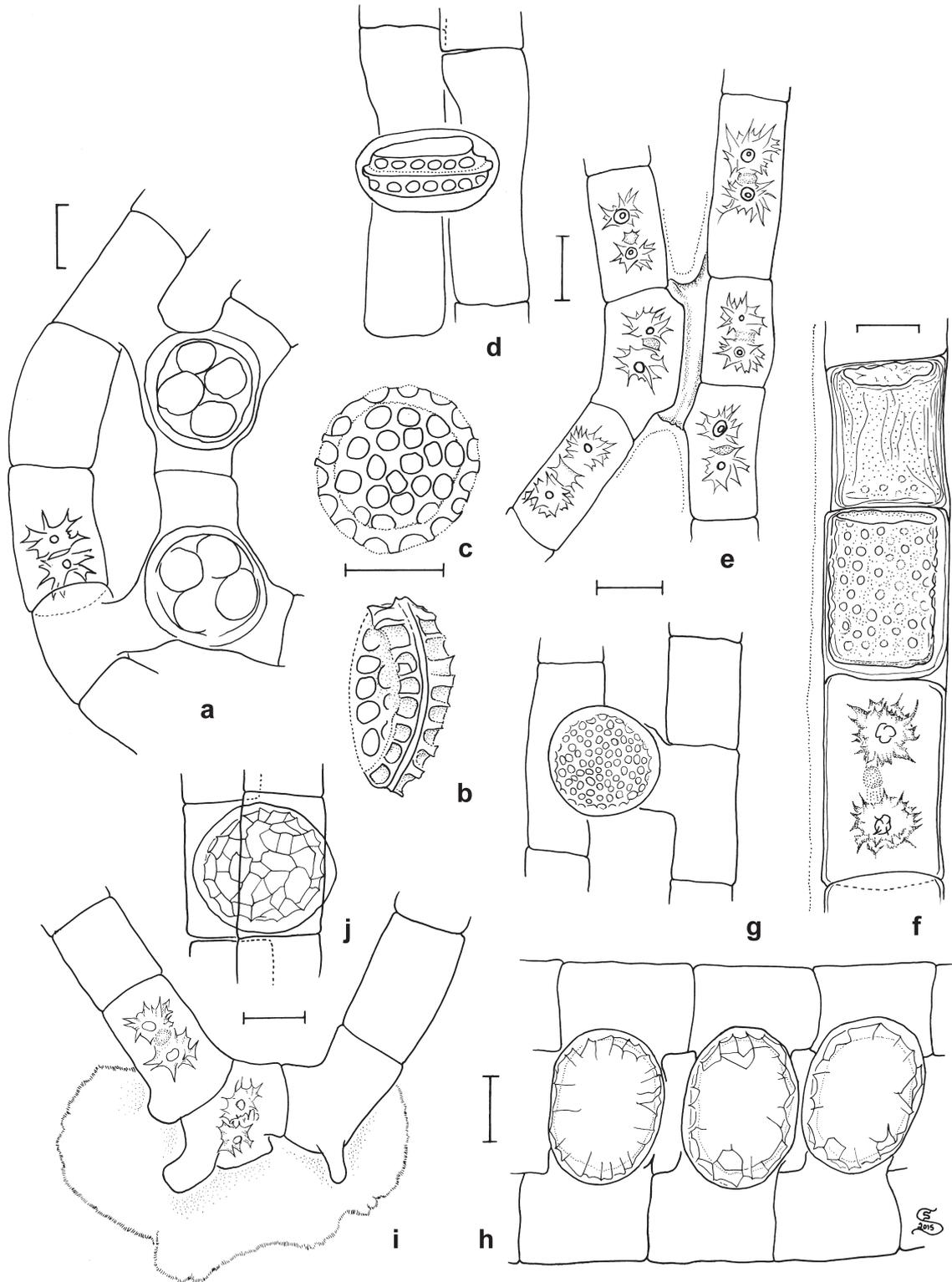
Earlier reports of *S. moebii* from Australia were considered doubtful by Lewis and Entwisle (2007).

The connexion of the conjugation tubes is fragile, and there were several runs of apomictic spores with domes on the lateral wall of the gametangial cell in *S. Skinner* 934. Bailey's (1895) transcription of Moebius' notes includes a reference to such irregularities in the Type collection.

*S. Skinner* 934 has similar dimensions to other descriptions in the literature. The number of chloroplasts in most descriptions is 6–8. In *S. Skinner* 896 the cell dimensions (66–75  $\mu\text{m}$  diameter for vegetative cells) are slightly reduced and the zygospore diameter (72.5–90  $\mu\text{m}$ ) a little large, but the major variation is in the number of chloroplast ribbons, of 3–6, with 3 being the most frequent. Both these collections have zygospores (and aplanospores) with very similar sculpturing.



**Fig. 3.** **a** *Spirogyra hassallii*, lateral conjugation (Grassy Creek, ACT, S. Skinner1005 and L Johnston); **b,c** *S. maxima* var. *minor*, **b**, detail of sculpturing of mesospore, **c**, zygospore in receptor (Freshford Creek, ACT, S. Skinner934 and L. Johnston); **d**, *Spirogyra* sp. aff. *S. multistrata*, conjugation with mature zygospore (Conder Wetlands, ACT, S. Skinner928); **e,f** *S. amplexans*, lateral conjugation and zygospore from above (Bradleys Creek, Googong Foreshore, NSW, S. Skinner1011 and A M Hoefler); **g, h** *S. chenii*, **g**, scalariform conjugation, **h**, lateral conjugation (Burra Creek, NSW, S. Skinner972); **i** *Spirogyra* sp. aff. *S. reflexa*, scalariform conjugation with mature spores (Queanbeyan River, Doeberls Point, NSW, S. Skinner 1021 and J-M Crouch); **j, k** *S. caroliniana*, **j**, scalariform conjugation, **k**, zygospore from above (Murrumbidgee River, Angle Bend, ACT, S. Skinner900 and L. Johnston). Scale bars for **a, c-k** 20µm; **b**, 10 µm.



**Fig. 4** a,b,c *Zygnema* sp. aff. *Z. subfoveolatum*, a. scalariform conjugation, b,c. mature zygospores, lateral and top view (retention dam, Mt Taylor, ACT, S. Skinner 888); d,e *Zygnema* sp. aff. *Z. synadelphum*, d. mature zygospore, e. attachment cushion between filaments (dam at head of Woolshed Creek, ACT, S. McArdle-English and A Westcott S. Skinner959); f. *Z. subcylindricum*, intercalary akinetes (Burra Creek, NSW, S. Skinner1041, P Duffy, B Geikie); g. *Zygnema* sp. aff. *Z. lenticulare*, zygospore (Frog Pond No 2, Mulligans Flat Nature Reserve, ACT, S. Skinner 893); h,i,j. *Zygnema chungii*, h. scalariform conjugation, i. attachment cushion for filament, j. zygospore top view (Queanbeyan River, Doeberls Point, NSW, S. Skinner 1021 and J-M Crouch). All scale bars 20µm.

### The curious case of the name '*Spirogyra moebii*'

Here is Moebius' (1894, 334–335) protologue, the contents of square brackets are my interpolations:

51. *Sp[irogyra] maxima* (Hassal) Wittr[ock]. var. *minor* nov. var.

[Type:] Dalby, Darling Downs, Queensland, pond. May 1893.

*A variety in which the vegetative cells are 78–80 μm thick, 2–3 times longer than broad, and with an 80 μm long (sic) [diameter] zygote.* [from the Latin]

*The present alga can be distinguished from the typical form by means of the slimmer cells and smaller spores, and, as it conforms in the remaining characters, it can be seen as a new variety. In the typical form the vegetative cells are 77–160 μm, mostly 132–138 μm, thick and hardly longer, the same size or actually shorter than the diameter, (and) the spores 102–115 μm long and 77–84 μm thick. [In this new variety] here we have cell and spore measurements as described above. The cells have 6–8 chlorophyll-bands making ½–1 turn. The receptor is not swollen, but shorter than vegetative cells. The spores are lenticular (discoid) and depending on aspect seen as circular or ellipsoidal, with, at maturity, a golden brown membrane. In copulation repeated anomalies occur, of which I will describe one here. The copulation tube from one filament may meet the cross-wall in another filament, and the cells on either side of the cross-wall will produce a conjugation tube, and the resulting three tubes become coalescent with one-another, sometimes with outgrowths. Without some membrane [cell-wall] re-absorbance, it would be hard to establish which two cells are actually copulating.*

*Sp. maxima* is known from Europe, North America, Argentina and Uruguay. [from the German]

There is no iconotype of *S. maxima* var. *minor* but Pl IX figs 2–4 in Bailey (1895) are from Moebius' pencil drawings sent to Bailey. In the spores in fig. 2 there is no indication of the reticulate mesospore sculpturing described for *S. maxima* more generally. The specimen may well have been destroyed during the bombing of the Frankfurt Botanic Gardens during the World War II. The above notes agree with what is in Bailey (1895).

Moebius' concept of *Spirogyra maxima* is interesting, as his 'mostly 132–138 μm' cell diameter would fit in the middle of the range outlined in Borge 1913. Borge was the one to emphasise the reticulated pitting of the mesospore wall. Borge (1903) presented what he claimed were the first illustrations of mesospore sculpturing for *Spirogyra maxima*, in response to a comment by Lagerheim.

Neither Borge (1913) nor Czurda (1932) include reference to Moebius' variety in their *S. maxima* citations.

Transeau (1934) made the name change from *S. maxima* var. *minor* to *S. moebii* without a description, and stated that such a taxon fitted an alga 'recently collected by Taft in Oklahoma (USA).' Transeau is the source of much confusion, giving 1895 as the date for the Moebius description. This confusion is compounded by Transeau (1951) who cited Moebius (1892) *Flora*, page 421, which mistake is copied in Kadłubowska (1984) and Jao (1988).

Kolkwitz and Krieger (1944) who cited Moebius correctly, provided figs 667 and 668 for *S. moebii*, and credit them to Borge. Those figures appear to be redrawn, reversed, from figures 18 and 21a for *Spirogyra maxima* in Borge (1903). Borge stated that fig. 18 was of the 'remarkably small zygosporos' in Malme's collection from Morrinho, Brazil, while the citation for fig. 21a is Erlangden, Bavaria, collected by Glück in August of 1888 (Borge, 1903, p. 285).

The Type specimen, the collection mentioned in the protologue, is missing.

The description in Moebius (1894) and Bailey (1895) does not mention sculpturing. The illustrations in Bailey (1895) do not provide clarity about sculpturing.

The renaming by Transeau (1934) has insufficient detail to confirm identity, and may only refer to the Oklahoma specimen with any certainty. The figures in Kolkwitz and Krieger (1944) are inappropriate.

Reversion to the Moebius citation appears the most appropriate course of action, until a suitable collection or set of collections can be made in Dalby and the surrounding Darling Downs, Queensland.

***Spirogyra megaspora*** (Lagerheim) Transeau, *Ohio Journal of Science* 34:420 (1934).

*S. maxima* (Hass.) Wittrock forma *megaspora crassa* Lagerheim in Wittrock & Nordstedt, *Algae exsiccatae* Nr. 745: 956(1883)

Vegetative cells shortly cylindrical, 174–189 μm diam., L/D 1.25–3, end-walls plane; 10–12 chloroplast ribbons, almost straight, numerous pyrenoids.

Conjugation scalariform; donor and receptor cells similar to vegetative cells; conjugation tube 'cup + socket' from donor; zygospore formed in receptor. Zygospore round to ovoid lenticular, 145–160 μm diam., c. 20 μm

thick; exospore smooth, lamellate, clear 2.5 µm thick; mesospore two layered, outer reticulate, inner finely pitted, chocolate brown.

**Distribution:** Europe and South America, newly reported for Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: marsh, John Reserve (north of archery butts), Holder, 23 May 2009 (S. Skinner 956).

Weston Creek, pH 7.0–8.1, 190–790 µS.cm<sup>-1</sup>; 10–15 NTU

The thin, almost straight chloroplasts are reminiscent of those in *Sirogonium sticticum*. Like *Sirogonium*, when handled this material was quite cartilaginous, and while silky, distinctly not greasy as many other silkweeds are. The very reduced socket of the receptor part of the conjugation tube is another morphological indication of similarity to *Sirogonium*.

Drummond et al. (2005) suggest strongly that DNA analysis supports the inclusion of the *Spirogyra maxima* complex within a more broadly defined *Sirogonium*. Hoshaw, Wells and McCourt (1987) demonstrate that material which fits the description of *S. megaspora* has 12 chromosomes, and is described as strain D of their *S. maxima* complex. With twelve or more already described taxa that could be included using morphology in an expanded *S. maxima*, it would serve little purpose to describe more until the genetics and physiology are better known. Stancheva et al. (2013) have shown that there may be two groups of species in *Spirogyra* with large numbers of chloroplast ribbons per cell, and that species presently assigned to *Sirogonium* may be found in one of these groups.

Further detailed examination of both *S. maxima* var. *minor* and *S. megaspora* may lead to their inclusion in not only an expanded *S. maxima*, but also transfer to the genus *Sirogonium* or, possibly more likely, *Sirogonium* being included in *Spirogyra*.

*Vegetative cells usually long (L/D >2), end-walls plane, with 4 (2–5) chloroplast ribbons Spirogyra B*

***Spirogyra* sp. aff. *dubia*** Kützing, *Species Algarum* 441(1849); Lewis and Entwisle *Zygnemaceae*: 125, fig. 39A (2007).

Type: probably not designated.

Vegetative cells cylindrical, 36–40 µm diam., L/D4–8, end-wall plane; 4 chloroplast ribbons. 2–3 turns, pyrenoids numerous.

Conjugation scalariform, receptor slightly inflated all round and then purse-like; conjugation tube funnel + socket, more from receptor side. Zygote ellipsoidal to cylindrical with rounded ends, 81–87(–119) µm long, 45–48 µm diam.; exospore smooth, pearly (laminated?); mesospore smooth, yellow brown (possibly immature).

**Distribution:** cosmopolitan; widespread in Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tableland: Giralang Pond, Giralang, 22 Oct 2007 (S. Skinner 957.3 & R. McConville).

pH 7.8–8.4, 230–390 µS.cm<sup>-1</sup>; 10–25 NTU

Close to the description in Lewis and Entwisle (2007) but as the material is not fully mature and the exospore appears to be laminated the distinction is preserved. This material is a candidate for narrower forms of a *S. neglecta* complex.

***Spirogyra neglecta*** (Hassall) Kützing, *Species Algarum* 441(1849); Kolkwitz & Krieger, *Rabenhorst's Kryptogamen-Flora*, 13, 2: 351, fig 491 (1944); Lewis and Entwisle, *Zygnemaceae*:132, fig 39C (2007)

*Zygnema neglectum* Hassall, *Annals and Magazine of Natural History* 10: 37(1842).

Type: Cheshunt and Tunbridge Wells, Kent, England. BM *n.v.*

Vegetative cells 61–67 µm diam., L/D 2.5–5(–6), end-walls plane; 4 chloroplast ribbons, 1.5–3 turns, pyrenoids numerous.

Conjugation scalariform; donor and receptor as vegetative cells; conjugation tube two funnels, from both gametangia. Zygospore ovoid ellipsoid, (78–)87–96(–102) µm long, 52–58 µm diam.; exospore clear, thin and smooth; mesospore two layered, outer layer clear, irregularly wrinkled, inner layer smooth, mid brown.

**Distribution:** Cosmopolitan; in tropical and temperate Australia.

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: farm dam, 607 Gum Lane, via Yass, 12 Nov 2012 (S. Skinner 980 & R. Lawton).

pH 7.5; 27 NTU; 100 µS.cm<sup>-1</sup>

The double layered mesospore is not noted in flora descriptions, but form and dimensions similar; this form is clearly part of the *S. neglecta* complex.

***Spirogyra punctulata*** Jao, *American Journal of Botany*, 23: 57, figs 13–14 (1936).

Type: western China

Vegetative cells cylindrical, (59–)64–71 µm diam., L/D 3–6, end-wall plane; 4 chloroplast ribbons, two turns, numerous pyrenoids. Rhizoids extending from some cells of some filaments.

Conjugation scalariform (or abortive); donor and receptor as vegetative cells; conjugation tube cylindrical, from both gametangia (closed on donor side if abortive). Zygospore (including autospore) ellipsoidal to cylindrical with rounded ends (triaxial ellipsoid?), 74–88 µm long, 52–57 µm diam.; exospore slightly pearly, thin and smooth; mesospore wrinkled, finely scrobiculate, rich brown.

**Distribution:** China, reported here for Australia.

**Specimen examined:** NEW SOUTH WALES: Southern tablelands: farm dam, *Talaheni*, via Murrumbateman, 23 Oct 2012 (*S. Skinner 978*). no water quality data

While there were some successful zygotes, and filaments had clearly paired for reproduction, the frequent autospores may have been induced by a chitrid or similar aquatic fungal infection, prominent in the collection. Like the previous taxon, this may very well represent a form within a *S. neglecta* complex. The general morphology conforms to descriptions in Kolkwitz and Krieger (1944), Jao (1988) and Kadłubowska (1984) but the diameter of the cells is slightly narrower. *Talaheni* is no more than 20 km south east of 607 Gum Lane and both farm dams were zoned wetlands with diverse biota showing minimal stock damage.

***Spirogyra* sp. aff. *multistrata*** Zheng & Ling, *Oceanographica Limnologica Sinicia* 10: 290, Pl 2, 5–6 (1979); Jao, *Zygnemataceae*, 101, Pl XXXVIII, 7–8 (1988).

Type: China, n.v.

Vegetative cells cylindrical, L/D 2.5–10 +, 29–33 µm diam., end-walls plane; 4 chloroplast ribbons, one complete turn; numerous pyrenoids.

Conjugation scalariform; donor cell as vegetative cell, shorter; receptor inflated on all sides, 43–52 µm diam., conjugation tube two funnels. Zygospore ovoid ellipsoid (biaxial ellipsoid), (74–)86–100(–109) µm long, 43–52 µm diam.; exospore thick, laminated, clear; mesospore wrinkled and with small pits, bronze brown. **Fig. 3d**

**Distribution:** China, reported here for Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: Conder Wetlands, Tom Roberts Drive, Conder, 4 Feb 2008 (*S. Skinner 928*).

pH 7.0–8.9, 100–320 µS.cm<sup>-1</sup>; median of 20 NTU

This taxon is narrower than the description for *S. multistrata*, otherwise similar. The biaxial ellipsoidal shape of the spore relates this taxon to *S. formosa* and *S. crassa*, but those taxa have numerous chloroplasts. The spelling follows Jao (1988).

Further research into these four morphological forms, especially DNA analysis, determination of ploidy levels and field and *in vitro* observations of the impact of environmental pressures, may place all of them in an expanded *Spirogyra neglecta* or *S. fluviatilis*. The comparison of field collections of morphospecies of *S. fluviatilis* in the Douglas and Daly Rivers in northern Australia (Schult et al. 2007) indicates the range of cell widths and chloroplast numbers that these species complexes can take, and how single collections are snapshots of moments in time.

*Vegetative cells frequently long (L/D >2), end walls plane, with a single chloroplast ribbon Spirogyra A.*

***Spirogyra chenii*** Jao, *Sinensia* 6: 587, pl 4, fig. 52 (1935).

Kadłubowska (1985).*Zygnemales*:283, fig 436. Lewis and Entwisle (2007) *Zygnemaceae*: 121, fig. 34B..

Type: Nui-Kioh-To, China. *CCJao5753*, Nov. 1934; syn: MICH. nv

Vegetative cells narrow cylindrical, (18–)23–26 µm diam., L/D 1.5–5(–6), end-wall plane; single chloroplast, 1–2(–2.5) turns, 6–8(–12) pyrenoids.

Conjugation lateral and scalariform, donor cell as vegetative cells, receptor distinctly swollen; conjugation tube in scalariform conjugation from the donor cell only, cylindrical. Zygospore (triaxially) ellipsoid, 39–50(–74) µm long, 23–29 µm diam.; exospore smooth and clear, mesospore smooth, golden or nut brown. **Fig. 3, g,h.**

**Distribution:** China, south eastern Australia.

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: Murrumbidgee R., floodrunner pool, de Salis Cemetery, Tharwa, 13 Dec 2007, (*S. Skinner 919* & *Luke Johnston*).

no water data, compare Angle Bend

NEW SOUTH WALES: Southern Tablelands: 3km downstream Burra Rd gauging station, Burra Creek, London Bridge, 15 May 2012 (*S. Skinner 972*); Williamsdale Road causeway, Burra Creek, Burra 18 Jan 2013 (*S. Skinner 999* and *W. O'Reilly*); swamp and ephemeral gutter, Doyles Reserve, Barracks Flat, Queanbeyan, 23 Oct 2013 (*S. Skinner 1012.2*); pond along railway line, Tralee, 29 Aug 2011 (*S. Skinner 965b*)

Burra Creek: pH 8.0–8.9; 430–600  $\mu\text{S}\cdot\text{cm}^{-1}$ ; c. <10 NTU. Queanbeyan River: pH 7.1–8.2; 90–200  $\mu\text{S}\cdot\text{cm}^{-1}$ ; c. <10 NTU.

Sterile filaments of comparable dimensions are frequently encountered in rivers and creeks in the region and may very well be referable to this taxon.

***Spirogyra lacustris*** Czurda, *Susswasserflora* 8: 176, fig 182 (1932)

Type: Lunz, Germany, BSR

Vegetative cells cylindrical, 37–38(–40)  $\mu\text{m}$  diam., L/D 2–4, end-wall plane; single chloroplast ribbon, 2–5 turns, numerous pyrenoids.

Conjugation scalariform or aborted; donor and receptor as vegetative cells; conjugation tube where completed funnel + tube. Zygospore (triaxial) ellipsoid, 59–62  $\mu\text{m}$  long, 26  $\mu\text{m}$  diam.; exospore thin smooth and clear; mesospore smooth, golden brown.

**Distribution:** widespread, reported here for Australia.

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: ford downstream of dam wall, Lower Tuggeranong Creek, Greenway, 2 Oct 2007 (*S. Skinner 892*);

NEW SOUTH WALES: Southern Tablelands: Jerrabomberra Creek, Tralee, 29 Aug 2011 (*S. Skinner 964*).

Lower Tuggeranong Creek: pH 7.8–9.1; 72–300  $\mu\text{S}\cdot\text{cm}^{-1}$ ; <10–20 NTU. Jerrabomberra Creek: pH 7.6–8.5, 210–360  $\mu\text{S}\cdot\text{cm}^{-1}$ ; <10–15 NTU.

This taxon may very well represent a local form in the *Spirogyra communis* complex. Many of the reproductive cells had unopened conjugation tubes, indicating autospore formation was more common than successful conjugation in this collection.

***Spirogyra* sp. aff. *reflexa*** Transeau, *Ohio Journal of Science* 16: 28 (1915)

Type: North America, Farlow, n.v.

Vegetative cells short cylindrical, 36–43  $\mu\text{m}$  diam., L/D 0.75–1.5, end-walls plane; single chloroplast, 1–1.5 turns, pyrenoids 6–8. Rhizoids at ends and bends in filaments.

Conjugation scalariform; receptor cells inflated inward, such that paired filaments bend, 58–59  $\mu\text{m}$  diam.; conjugation tube funnel from donor. Zygospore ellipsoid, perpendicular to filament, 57–59  $\mu\text{m}$  long, 29–32  $\mu\text{m}$  diam.; exospore clear, wrinkled 'like a walnut'; mesospore smooth, brown. **Fig. 3i**

**Distribution:** North America, reported here for Australia.

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: Doeberls Point, Queanbeyan R., Barracks Flat, Queanbeyan, 3 Feb 2014 (*S. Skinner 1021* & *J-M Crouch*)

pH 7.1–8.2; 90–200  $\mu\text{S}\cdot\text{cm}^{-1}$ ; c. <10 NTU.

***Spirogyra mirabilis*** (Hassall) Kützing, *Species Algarum* 438 (1849) forma. Lewis and Entwisle *Zygnemaceae*: 131, fig. 34A. (2007)

Vegetative cells cylindrical, 31–38  $\mu\text{m}$  diam., L/D 1.5–8 end-walls plane; single spiral chloroplast, 1–3 turns, 6–8+ pyrenoids.

Conjugation absent; autospores in long series. Conjugation tube, where present, an abortive dome. Autospore elliptical, sometimes oblique in generative cell, 45–55(–61)  $\mu\text{m}$  long, 24–35  $\mu\text{m}$  diam.; exospore clear, smooth and thin; mesospore smooth, thin, sometimes collapsed, yellow-brown to golden brown.

**Distribution:** cosmopolitan, reported from south-eastern Australia.

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tableland: Giralang Pond, Giralang, 22 Oct 2007 (*S. Skinner 957.3 and R. McConville*);

NEW SOUTH WALES: Southern Tablelands: pond along railway line, Tralee, 29 Aug 2011 (*S. Skinner 965*).

Giralang Pond: pH 7.8–8.4, 230–390  $\mu\text{S}\cdot\text{cm}^{-1}$ ; 10–25 NTU. No water quality data for Tralee.

One of four smooth spored taxa with autospore/parthenospore formation in Kolkwitz and Krieger (1944) and Transeau (1951) and eight in Kadłubowska (1984). *S. maravillosa* Transeau, with 2–3 chloroplast ribbons, has a similar morphology. *S. azygospora* Singh has five chloroplasts and lenticular spores and much wider cells, as does *S. wrightiana* Transeau, with 6–8 chloroplasts and ellipsoid spores. *S. gujaratensis* Kamat, with ellipsoid spores, and *S. kamatii* (Kamat) Kadłubowska, with lenticular spores, have 2–5 chloroplasts and are wider than *S. maravillosa*. The two other taxa in Kadłubowska (1984) have replicate end-walls.

These vegetative cells are slightly greater in diameter than those described in other publications (21–27(–33)  $\mu\text{m}$  in Kolkwitz and Krieger (1944), 21–28(–33)  $\mu\text{m}$  in Jao 1988). This may indicate that these collections are not genetically related to those in other parts of the world, or alternatively, the range of cell diameters needs to be broadened. Collections from Bool Lagoon in South Australia (Skinner 1986) have cells with 28–32  $\mu\text{m}$  diam. and those from Victoria in Lewis and Entwisle (2007) 23–33  $\mu\text{m}$  diam. and conform more closely to overseas material.

*Vegetative cells often very long and narrow (L/D > 3), end walls replicate, with a single chloroplast ribbon, occasionally two, Spirogyra S.*

***Spirogyra hassallii*** (Jenner) Petit, *Les Spirogyres des environs de Paris*, 13, pl 2 fig 6–7 (1880). Randhawa 1959 *Zygnemaceae*: 367, fig 396.

*Zygnema hassallii* Jenner, *Flora of Tunbridge Wells*, 182 (1845)

Type: Tunbridge Wells, United Kingdom. BM? (n.v.)

Vegetative cells cylindrical, 26–28(–29)  $\mu\text{m}$  diam., L/D 5–10+, end-walls replicate; single chloroplast ribbon, 2–4 open turns, pyrenoids numerous.

Conjugation both scalariform and lateral; receptor less than 20% inflation; conjugation tube funnel to funnel from both gametangia. Zygospore ellipsoid to cylindrical with rounded ends, 78–93(–154)  $\mu\text{m}$  long, (26–) 34–41  $\mu\text{m}$  diam.; exospore thin, smooth and clear; mesospore, thick, mid brown. **Fig. 3a.**

**Distribution:** northern hemisphere, newly reported for Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: Namadgi National Park: Boboyan Road bridge, Grassy Creek, 9 May 2013 (*S Skinner 1005 & L. Johnston*)

Lewis and Entwisle (2007) rejected previous records of this species for Australia.

The single chloroplast rather than two per cell is the main distinction between *S. Skinner 1005* and previous descriptions. The filament width and the conjugation tube from both sides suggest a possible relationship. This doesn't fit *S. hopeiensis* Jao, with similar filaments and spores, but conjugation tube from donor gametangia only.

***Spirogyra caroliniana*** Dillard, *Phycologia* 7: 1, fig.1 (1969). Kadłubowska, *Susswasserflora Mitteleuropa*, 16: 449, fig 699(1984)

Type: spring pool, Millwee Creek, South Carolina (USA). US National Herbarium, GD-3-2867-2a. n.v.

Vegetative cells cylindrical, 23–28  $\mu\text{m}$  diam., L/D 2.5–>10, end-walls replicate; single chloroplast ribbon, 2–3 turns, pyrenoids 15–20. Filaments attach to substrate with mucilage plugs.

Conjugation scalariform; receptor inflated, forming cylinder around the zygospore; conjugation tube from donor cell. Zygote ellipsoid, (49–) 61–73  $\mu\text{m}$  long, 29–35  $\mu\text{m}$  diam.; exospore thin, smooth and clear; mesospore two layered, smooth, rich brown. **Fig. 3j, k**

**Distribution:** South Carolina, USA, newly reported for Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: riffle, Murrumbidgee River, Angle Bend, 15 Nov 2007 (*S. Skinner 900 and L. Johnston*)

pH 8.2; 22 NTU; 71  $\mu\text{S}\cdot\text{cm}^{-1}$

The cylindrical swelling of the receptor and the conjugation tube developing from the donor, place this collection in *S. caroliniana*.

***Spirogyra* sp. aff. *farlowii*** Transeau, *Ohio Journal of Science* 16: 29 (1915). Lewis and Entwisle *Zygnemaceae*: 125, fig. 41 C, D (2007).

Type: probably not designated.

Vegetative cells cylindrical, 25–26 µm diam., L/D 2–8, end-walls replicate: chloroplast ribbons 1(–2), 5–6 turns, pyrenoids numerous.

Conjugation scalariform, receptor inflated towards the conjugation tube; conjugation tube mainly from receptor. Zygospore more or less cylindrical with rounded ends, c. 76 µm long, c. 36 µm diam.: exospore thin, smooth, clear; mesospore smooth, thick, brown.

**Distribution:** widespread, south-west and south-east Australia.

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: farm dam, Tin Hut Dam Creek watershed/catchment, London Bridge Road, Burra, 10 Sep 2013, A. Jones (*S. Skinner 1009.6*)

no water quality data

There is no other taxon in this section of the genus where the conjugation tube is derived only from the receptor.

It is possible that the form of the conjugation tube may be as plastic a character as vegetative cell width and possibly form and degree of inflation of receptor and the smooth spored, replicate end-walled *Spirogyra* species may form one or a small number of species complexes.

***Spirogyra amplexans*** Skuja, *Algae in Handel-Mazzetti, Symbolae Sinica* 1: 85, figs 9–11 (1937).

Type: Szechwan Province, China, 4100m altitude. (n.v).

Vegetative cells cylindrical, 15–25 µm diam., L/D 3–10+, end-walls replicate; single chloroplast ribbon, 2–4 turns, pyrenoids 6–10.

Conjugation lateral, rarely scalariform; receptor cell bulging inflation; conjugation tube in lateral conjugation slightly larger on donor side. Zygospore ovoid ellipsoid, 55–88(–95) µm long, 26–40 µm diam.; exospore thin, clear; mesospore two layers, upper reticulate, inner with irregular pits, golden brown. **Fig. 3e, f**

**Distribution:** China, newly reported from Australia.

**Specimen examined:** NEW SOUTH WALES: Southern Tablelands: Bradleys Creek, Googong Foreshores, ford at end of fire trail, 20 Oct 2013 (*S. Skinner 1011 and A-M Hoefler*).

no water quality data

The Bradleys Creek sample is close to the species as described and illustrated in Kolkwitz and Krieger (1944) Randhawa (1959) and Jao (1988) except that the exospore appears smooth rather than finely papillose.

***Zygnema*** C.Agardh, *Systematica Algarum* 77 (1824)

Type: *Z. cruciatum* (Vaucher) C.Agardh

*Zygospores formed in the receptor*

***Zygnema*** sp. aff. *subfoveolatum* Chian f. *mengtzensis* Chian, *Acta Phytotaxonomica Sinica* 19: 131 (1981)

Type: Lake Nanhu, near Mengte, Yunnan, China, April 1974. YN:7401.

Vegetative cells shortly cylindrical, 22.5–24 µm diam., L/D 1.5–3, end walls plane; two stellate chloroplasts.

Conjugation scalariform; zygospore formed in receptor, encapsulate. Outer spore wall (capsule) clear, disappears with maturity; mesospore flat-topped discoid, ± circular in face view, 33–38 µm diam., 16–18 µm thick, with a central lip on rim; sculpturing of deep, large, quadrate scrobiculae, 5–7 µm diam., golden brown. **Fig. 4a–c**

**Distribution:** China, newly reported for Australia.

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: retention dam, head of Weston Creek, Waldock St, Mt Taylor, 5 Sep 2007 (*S. Skinner 888*), 31 Oct 2007 (*S. Skinner 898*).

This taxon is very close to the Chian (1981) forma, but also has some resemblance to *Z. reticulosporum* Gauthier-Lièvre as described in Kadłubowska (1984).

*Zygospores formed in the conjugation tube*

***Zygnema*** sp. aff. *lenticulare* Jao & Hu, *Oceanographica Limnologica Sinica* 9:195, plate 1, figs 8–9 (1978)

Type: China. n.v.

Vegetative cells cylindrical, 26–27 µm diam., L/D (1.5–)3–4, end walls plane; two stellate chloroplasts.

Conjugation scalariform; Zygosporangium in conjugation tube, encapsulate. Zygosporangium depressed globose; outer wall (capsule) clear, retained at maturity, 30–35  $\mu\text{m}$  diam.; mesospore scrobiculate, dimples shallow, moderately large (2.5–3.5  $\mu\text{m}$  diam.); dull blue. **Fig. 4g**

**Distribution:** China, newly reported from Australia.

**Specimen examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: Frogpond No. 2, Mulligans Flat Reserve, Forde, 10 Oct 2007 (*S. Skinner 893*).

This taxon is clearly in the *Z. coeruleum* Czurda group, and is similar to *Z. porcatum* Jao & Hu but does not have a rim or an aereolate capsule.

*Zygnema* sp. aff. *synadelphum* Skuja, *Acta Hortus Botanicus Universitas Latviae* 1: 109, Pl 1, fig.2a (1926).

Type: Latvia. UPS n.v.

Vegetative cells cylindrical, (17–) 21–26  $\mu\text{m}$  diam., L/D 5–7, end-walls plane; two stellate chloroplasts. Filaments may form a loose reticulated matrix, with mucilage pads and knees in the filaments at junctions.

Conjugation scalariform; zygosporangium formed in conjugation tube and partly in receptor, encapsulate. Zygosporangium discoid, with a raised median rim 2–3  $\mu\text{m}$  wide, face view ovoid to chordate, (28–) 33–38  $\mu\text{m}$  diam. inside rim. Mesospore scrobiculate, large dimples 2.5–3.5  $\mu\text{m}$  in a radiating pattern; royal blue. **Fig. 4d, e**

**Distribution:** cosmopolitan, name may appear in environmental reports for Australian locations.

**Specimens examined:** AUSTRALIAN CAPITAL TERRITORY: [NEW SOUTH WALES] Southern Tablelands: spring-fed farm dam at head of Woolshed Creek, Majura Valley, 31 Aug 2010 *S. McArdle English & A. Westcott*, (*S. Skinner 959*).

pH 7.5–8.0; 70–170  $\mu\text{S}\cdot\text{cm}^{-1}$ ; <10–20 NTU

The net-like habit of the filaments in the Woolshed Creek specimen may be a response to local water conditions. The material was suspended as barely submerged rafts and the array may have increased the Reynolds Number sufficiently to hold the filaments in suitable position for photosynthesis without gas bubbles. Stancheva et al. (2012) report similar knees in *Z. californicum* Stancheva, J.D.Hall and Sneath where the filaments met the substrate. The cell diameter is a little narrow for the description in Kolkwitz and Krieger (1941).

The *Zygnema coeruleum* group may also be a species complex, and the above two taxa may turn out to be morphological forms within the one genetic taxon.

*Zygnema chungii* Li, in Transeau, Tiffany, Taft & Li, *Transactions of the American Microscopical Society* 53: 213, pl 18, fig 15 (1934); Jao 1988, *Flora Algarum Sinicarum Aquae Dulcis*, 1: 8, pl 2, fig 1.

Type: Wuchang, Hupeh, China, (*H.H. Chung A1220 & F294*).

Vegetative cells short cylindrical, 24–26(–27)  $\mu\text{m}$  diam., L/D 1.25–1.75(–2.0), end-walls plane; two stellate chloroplasts. Filaments may form peg-like rhizoids surrounded by mucilage pads on pebbles of waterway base.

Conjugation scalariform; Zygosporangium formed in conjugation tube and backfilling into both gametangia, encapsulate. Zygosporangium globose to subcylindrical, 38–45  $\mu\text{m}$  long, 38–44  $\mu\text{m}$  diam., 32–33  $\mu\text{m}$  thick; exospore (capsule) clear, smooth; mesospore two layered, outer layer aereolate, inner layer finely papillose, yellow. **Fig. 4h–j**

**Distribution:** China, newly reported from Australia.

**Specimens examined:** NEW SOUTH WALES: Southern Tablelands: Queanbeyan River, Doeberl's Point, Barracks Flat, Queanbeyan, 3 Feb 2014 (*S. Skinner 1021*), 18 Feb 2014 (*S. Skinner 1025*).

pH 7.1–8.2; 90–200  $\mu\text{S}\cdot\text{cm}^{-1}$ ; c. <10 NTU.

While generally fitting the descriptions in Transeau et al. (1934) and Jao (1988) the scrobiculae reported in those collections appear as minute papillae in the Queanbeyan River material.

*Akinetes formed without conjugation or subsequent zygosporangium formation*

*Zygnema subcylindricum* Krieger, in Kolkwitz & Krieger, *Rabenhorst's Kryptogamenflora*, XIII: 262, Figs 330–331 (1941).

*Zygnema cylindricum* Transeau sensu Czurda (1932)

Type: Stellen, Czech Republic (Czurda), lost?

Vegetative cells short cylindrical, 33–38  $\mu\text{m}$  diam., L/D 1–3, end-wall plane, two stellate chloroplasts with a single, occasionally paired, pyrenoid per chloroplast.

Conjugation unknown in present material. Akinete (autospore) cylindrical, filling the cell, (45–)50–59(–81)  $\mu\text{m}$  long, 32–36  $\mu\text{m}$  diam.; exospore clear, often with a wrinkle; mesospore thick, laminated, with fine pitting as well as shallow dimples (c. 2.5  $\mu\text{m}$  diam.), golden brown. **Fig. 4f**

**Distribution:** north Germany and the Czech Republic in Europe, also California, USA; newly reported for Australia.

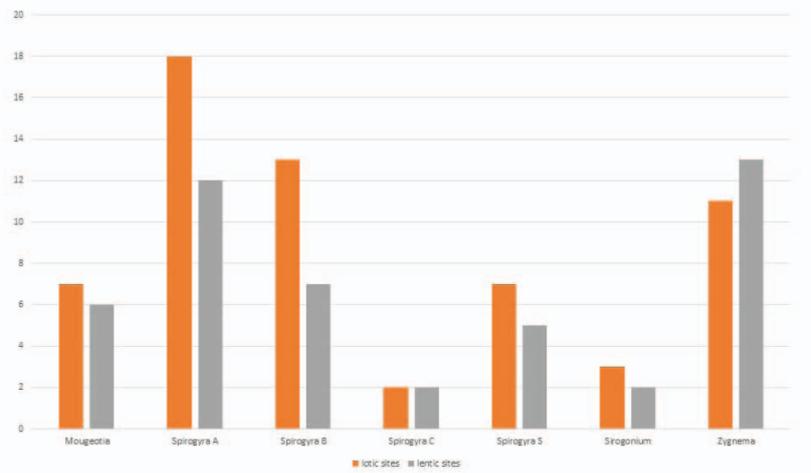
**Specimens examined:** NEW SOUTH WALES: Southern Tablelands: Burra Creek, junction of Williamsdale and Burra roads, Burra, 25 November 2008, (S. Skinner1041, P. Duffy and B. Geikie)

pH 8.0–8.9; 430–600  $\mu\text{S}\cdot\text{cm}^{-1}$ ; mode of <10NTU.

Dimensions are slightly larger than those given for European material.

## Part 2: Biogeography

Here is a snapshot of the potential diversity in the Zygnemataceae in the Upper Murrumbidgee River Catchment flora (see Appendices 2 and 3 for details). The majority of sites had a single taxon or two taxa present, but some sites had as many as five or six different morphological entities present (Fig. 4). Of the 28 lotic sites, the majority (19) had one or two silkweed taxa only, although one site (Burra Creek between the gauging station and London Bridge homestead) had six taxa. Of the 21 collections from lentic sites with Zygnematales present, seven have only a single taxon, and five have two taxa, while nine have three or more.



**Fig. 5.** Numbers of taxa of silkweeds at flowing (lotic) and standing (lentic) aquatic sites in the upper Murrumbidgee catchment.

**Table 2.** Profile of the Zygnemataceae collected in the Upper Murrumbidgee River Catchment, 2007–2013.

	No. Collecting Sites	No. of vegetative strains by cell diameter	No. showing reproduction	Range of morphological strains per site
<b>Lotic or moving water sites</b>				
<i>Mougeotia</i>	7		1	1–2
<i>Sirogonium</i>	2	1	1	1
<i>Spirogyra C</i>	2	2	1	1
<i>Spirogyra B</i>	13	2	1	1–2
<i>Spirogyra A</i>	17	3	4	1–2
<i>Spirogyra S</i>	7	3	3	1–2
<i>Zygnema</i>	12		2	1
<b>Lentic or standing water sites</b>				
<i>Mougeotia</i>	6		1	1
<i>Sirogonium</i>	1	1	1	1
<i>Spirogyra C</i>	1	1	1	1
<i>Spirogyra B</i>	5	4	3	1–3
<i>Spirogyra A</i>	10	3	2	1
<i>Spirogyra S</i>	4	2	1	1–2
<i>Zygnema</i>	9		3	1–2

The difference in number of cell diameter categories and the number of reproductive taxa indicates the difficulty in species delimitation, especially in the absence of reproductive material in a collection. There are four species of *Spirogyra* A (forms with one chloroplast) in flowing systems on the basis of their reproductive characters, but only three forms based on cell dimensions. Similarly, in *Spirogyra* B (with two to four chloroplasts) found in wetlands there are more forms than there verified species. In both *Spirogyra mirabilis* and *Zygnema subcylindricum* reproductive structures are formed without conjugation, and the vegetative dimensions of these taxa may overlap with sterile material in other collections, as well as with conjugating taxa.

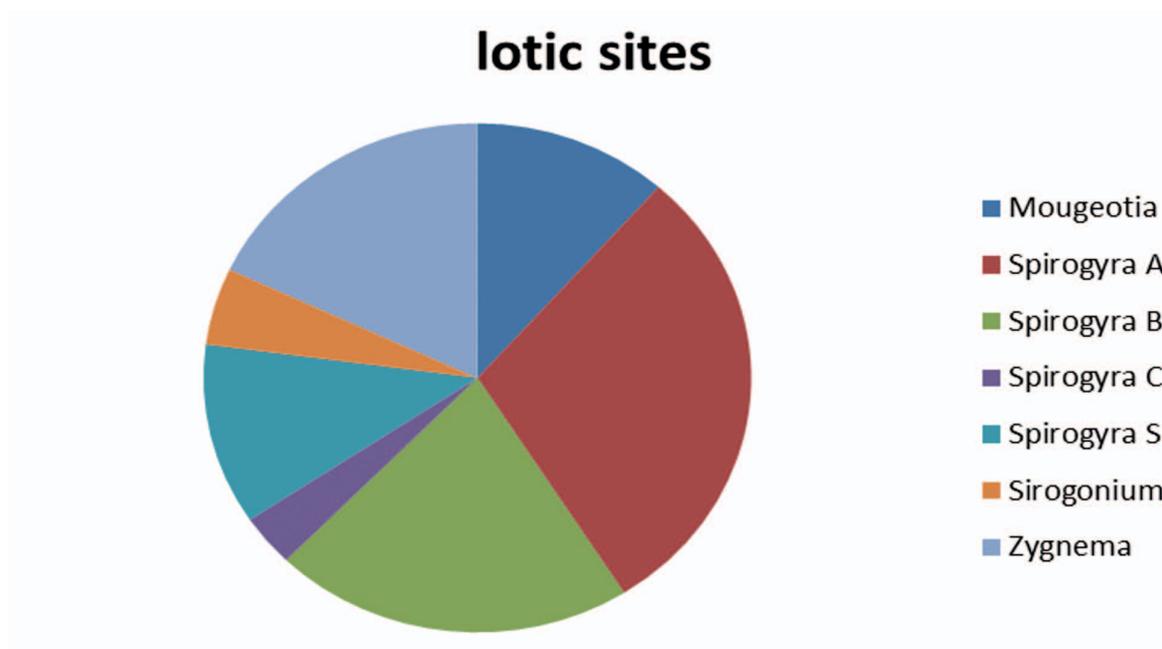


Fig. 6. Diversity of silkweeds in lotic waterways in the Upper Murrumbidgee River catchment.

In flowing water collections across the upper Murrumbidgee River catchment 66 % of silkweed taxa were forms of *Spirogyra*, of which most were either single chloroplast (A) or 2–6 chloroplast (B) plane end-wall taxa. *Zygnema* species were the next most common, as frequently encountered as single chloroplast *Spirogyra* species. *Spirogyra* with 6 or more chloroplasts and plane end-walls (C) were infrequently encountered, at about the same frequency as *Sirogonium* species. As elsewhere replicate end-wall species (S) of *Spirogyra* are about as common as *Mougeotia*.

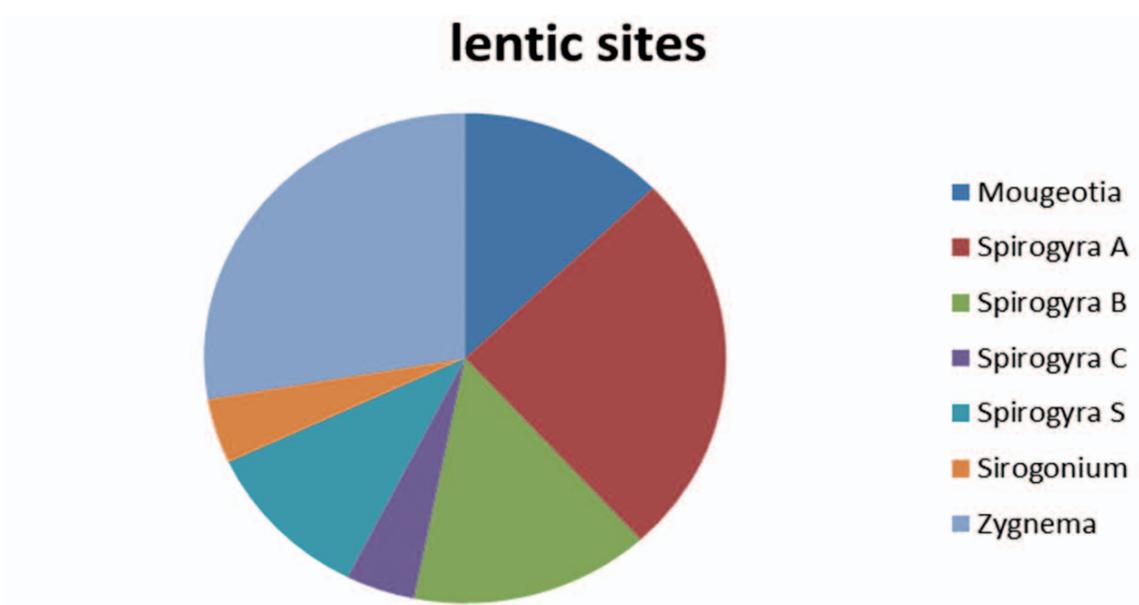


Fig. 7. Diversity of silkweeds in lentic waterways in the Upper Murrumbidgee River catchment.

*Spirogyra* species made up 55% of all taxa reported in wetlands, half of which were species with a single chloroplast and plane end-walls. *Zygnema* species made up 28% of the taxa, again with similar frequency of occurrence as the single chloroplast, plane end-wall *Spirogyra* species (26%). *Spirogyra* species with 2–6 chloroplasts and plane end-walls were less frequently encountered in lentic habitats.

## Discussion

The recent publication of a flora style review of Zygnemataceae in Australia (Lewis and Entwisle 2007) and the review of past records that preceded it (Lewis and Entwisle 1998) provide a well-documented background against which a paper like this can be viewed. Of the twenty two taxa described above, six are also described in Lewis and Entwisle (2007), one was regarded by them as a doubtful record, and two were rejected as incorrectly identified. The remaining thirteen constitute new records for Australia.

*Mougeotia lamellosa* appears to be a valid determination, but may turn out on further investigation to belong to a more broadly described *M. robusta* (de Bary) Wittrock. *Mougeotia* was poorly represented in Lewis and Entwisle (2007), as many early records were difficult to verify. Recent collections from across the continent will be used to demonstrate that the genus is well represented here (Skinner and Entwisle, in prep.)

*Sirogonium sticticum* is reinstated here, as most of the material collected in the Upper Murrumbidgee Catchment fits Hoshaw's (1980) description of that entity. If Lewis and Entwisle (2007) and Hoshaw (1980) are followed strictly there are two taxa in *Sirogonium* sensu Hoshaw in the Upper Murrumbidgee catchment. The one found in flowing water would fit *S. floridianum*, while the one from swampy ground *S. sticticum*. The distinction on width of vegetative cells seems arbitrary without molecular biological support.

There have been numerous attempts to discriminate between groups of taxa within the genus *Spirogyra*. Reproductive material has been separated out on cell end-wall structure, then number of chloroplast ribbons, then on presence or absence of sculpturing on the mesospore of the zygote. The reliance on morphology alone has been demonstrated to be inconclusive, not only from the chromosomal work of Godward and her students (Godward 1966) but again, and including physiological, breeding and biogeographical approaches of Hoshaw, McCourt and their group (Hoshaw and McCourt 1990) and the molecular studies of Drummond et al. (2005). Stancheva *et al.*, (2013) presents a more complex picture using rather larger numbers of species, with traditional groups dispersed across the genus, at the molecular level.

Especially with field collections of vegetative only material the classical sections of the genus fail. The vegetative groups outlined in Lewis and Entwisle (2007) based on the arbitrary separation of taxa as having cell diameters of more or less than 50µm is useful in a flora treatment. The present treatment removes the arbitrary width and distinguishes groups from field material on end-wall form and chloroplast numbers. Under a dissecting microscope the end-wall can be determined and the chloroplast number counted: one chloroplast *Spirogyra* A, two to four chloroplasts *Spirogyra* B, six or more chloroplasts *Spirogyra* C.

The case for the reinstatement of *Spirogyra maxima* var. *minor* is made above at length. All four of the separately described taxa in *Spirogyra* B may well be parts of the same or parallel polyploid series in related morphospecies. *Spirogyra punctulata* has larger all round dimensions than any similar taxa in Lewis and Entwisle (2007) and *S. multistrata* has laminated exospores, distinct from *S. schmidtii* West & G.S. West as described in Lewis and Entwisle (2007). *Spirogyra chenii*, reported from one locality in Lewis and Entwisle (2007) is the most widely and frequently encountered taxon in this catchment.

The *Spirogyra* S taxa in Lewis and Entwisle (2007) do not include any species with mesospore sculpturing, so the report of *S. amplexans* is new for the continent.

*Zygnema* species are frequently difficult to discriminate taxonomically. Again, the protologues and illustrations are often inadequate by present standards. Filaments with two stellate chloroplasts per cell were distinctly better represented in still waters than in creeks and rivers, although *Zygnema chungii* does seem to thrive in the Queanbeyan River in the built-up areas. The collections presented here give taxa with morphologically distinct characters, and in each case have some similarities to already described taxa. None of them fit precisely with either Eurasian or Western Hemisphere taxa. Many of the taxa to which our taxa can be aligned are poorly, incompletely or imprecisely described. The two blue spored *Zygnema* species described here replace similar taxa reported as doubtful in Lewis and Entwisle (2007) and there have been no previous descriptions of autosporic *Zygnema* in Australia.

Because of the haphazard pattern of collecting for this report, there is insufficient rigour to allow more than a sketch of the biodiversity of silkweeds in the Upper Murrumbidgee Catchment here. There are few distinct differences in floristics between these lotic and lentic collections from the Upper Murrumbidgee Catchment.

A more rigorous study with repeated site sampling, culturing and DNA labelling of vegetative material may change this view. Most collections of silkweeds are not reproductive and include a range of filament widths and chloroplast forms. The constituents of a raft from one locality may include representatives of several genera, and especially in *Spirogyra*, there may be more than one morphospecies from a species complex. This was very much the case in the Douglas-Daly river system in northern Australia (Schult *et al.* 2007).

Even so, the overall biogeographic picture has similarities with studies in Spain, and in part in the Netherlands and Arizona. Cambra and Aboal (1992) note tendencies for Zygnemaceae to dominate standing water both in diversity and bulk, and to be well represented in flowing water in Spain. Moreno *et al.* (2001) found that Zygnemales were well represented in ephemeral waters in semi-arid parts of Spain, more similar to inland rangeland in Australia. While *Spirogyra* was the most frequently encountered genus of the Zygnemales, two species of *Zygnema* were the most frequently found species in that part of Spain (Moreno *et al.* 2001). These two, and a small number of *Spirogyra* species were salt tolerant enough to procreate in conductivities approaching  $3.7 \text{ mS}\cdot\text{cm}^{-1}$ . In the Netherlands, similar observations were made (Simons 1987, Simons and van Beem 1990) while studying sporing periodicity and water quality influences on Zygnemales.

There are numerous studies to indicate the plasticity of morphology in silkweeds, based on external stimulants as well as ploidy level. For instance Berry and Lembi (2000) while examining seasonal variation in biodiversity also demonstrated that numerous temperature and light related parameters contributed to morphology of both individual filaments and mat cohesiveness in *Spirogyra*. McCourt, Hoshaw and Wang (1986) travelled North America collecting, and found *Spirogyra* turned up much more frequently than either *Zygnema* or *Mougeotia*, and that many collections showed several strains of *Spirogyra* with plane end walls, while single strains of replicate end-walled *Spirogyra*, *Zygnema* and *Mougeotia* were usually present, when these were encountered. Wang, Hoshaw and McCourt (1989) examined a suite of *Spirogyra* strains in a comparative climatic region of Arizona to the ACT region and found connexions between cell morphology and factors like altitude and bank cover. In Bear Creek, *S. quadrilaminata* Jao, which would belong in the *Spirogyra* B morphological group here, was found to be of common occurrence and sometimes dominant in all three sections of the waterway (Wang, Hoshaw and McCourt 1989). As only one site on Tuggeranong Creek, below the spillway, was repeatedly sampled comparisons are difficult to make (Skinner 2008).

Hainz *et al.* (2009) demonstrate that water chemistry is significant in occurrence of morphotypes of *Spirogyra* in different ecological niches, especially total phosphorus, soluble reactive phosphorus and organic nitrogen. Collection of similar data when collection of *Spirogyra* and other silkweeds are made in Australia may help build a similar basis for morphotype definition for Australian conditions.

Miller and Hoshaw (1974) indicate that cell and filament width may be reflective of ploidy level and is a very plastic character in *Zygnema circumcarinatum* Czurda.

The silkweed flora of the Upper Murrumbidgee River Catchment is species rich and shows much the same ecological behaviour as similar parts of the world. Similar species richness and distribution of morphological groups may be found in the headwaters of the other river systems that contribute to the Murray–Darling Basin.

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### Appendix 1: Water Quality Data:

These data were supplied by Upper Murrumbidgee Waterwatch, whose community scientists collect these, and several other parameters, on a monthly basis. Some collections, at locations not monitored by Waterwatch, had water quality data taken at the time of collection and these have been included with the locality data. Several sites did not have water quality data taken.

Catchment	Locality	pH range	Electrical Conductivity range ( $\mu\text{S.cm}^{-1}$ )	Turbidity range (NTU)
Gudgenby-Naas	Naas River at Namadgi National Park fence (for Grassy Creek)	6.6–8.0	53–90	<10–10
	Orroral River at the camp ground ford	7.0–7.5	50–95	<10–15
Murrumbidgee in ACT	Conder Wetlands	7.0–8.9	100–320	median of 20
	Murrumbidgee River at Pine Island (for Red Rocks Gorge)	7.8–8.7	75–160	<10–20
	Lower Tuggeranong Creek	7.8–9.1	72–300	<10–20
Queanbeyan	Burra Creek	8.0–8.9	430–600	mode of <10
	Queanbeyan R. at Doeberls Point	7.1–8.2	90–200	mode of <10
Molonglo	Woolshed Creek headwaters	7.5–8.0	70–170	<10–20
Jerrabomberra	Jerrabomberra Falls (for Tralee)	7.6–8.5	210–360	<10–15
Ginninderra	Giralang Pond	8.0–8.4	230–390	10–25
	East Basin, Lake Ginninderra	7.1–8.3	140–320	<10–56

### Appendix 2: Numbers of taxa recorded at lotic sites.

Locality	Mougeotia	Spirogyra, 1 chl. p	Spirogyra, 2+ chl., p	Spirogyra 'maxima'	Spirogyra Salmacis	Sirogonium	Zygnema	Total
Tuggeranong Creek		1						1
Giralang Pond		2	1					3
John Knight Memorial Park Creek	1			1	1		1	4
Murrumbidgee River, Angle Bend	1				1			2
Murrumbidgee River, Angle Bend		1						1
Orroral River						1		1
Murrumbidgee River, Tharwa		1			2		1	4
Murrumbidgee River, 'Lanyon'		1	1					2
Murrumbidgee River, Point Hut Crossing			1					1
Murrumbidgee River, near Pine Island							1	1
Tuggeranong Creek		1						1
Murrumbidgee River, Red Rocks Gorge		1					1	2
Freshford Creek				1			1	2
Cotter River, Campground		1	1					2
New Station Creek						1		1
Gibraltar Creek		1						1
Numeralla River, Mt Forest Road		1	1					2
Numeralla River, Chakola			1					1
Jerrabomberra Creek, Tralee		1						1
Burra Creek, Burra Road bridge	1	1	1		1		1	5
Burra Creek, 3km downstream	2	1	1		1		1	6
Molonglo River,	1							1
Burra Creek, Williamsdale Road		1	2				1	4
Grassy Creek	2		1		1		1	5
Bradleys Creek	1		1		1		1	4
Queanbeyan River, Doeberls Point		1					1	2
Queanbeyan River, Doeberls Point		1	1					3
Totals	7	18	13	2	7	3	11	

**Appendix 3: Numbers of taxa recorded at Lentic Sites**

Locality	Mougeotia	Spirogyra, 1 chl <sub>p</sub> , p	Spirogyra, 2+ chl <sub>p</sub> , p	Spirogyra 'maxima'	Spirogyra Salmacis	Sirogonium	Zygnema	Total
Mt Taylor dam		1			1		2	4
Mt Taylor dam		1			1		2	4
Mt Taylor dam	1	1					1	4
Pialligo, nursery						1		1
Mulligans Flat, pond 2	1				1		1	3
Mt Taylor dam							1	1
Mt Taylor dam							2	2
Conder Wetlands	1	1	1	1			2	5
Conder Wetlands			1					1
John Park, Holder					1			1
Head of Woolshed Creek, dam							1	1
North Watson Wetlands		1	3					4
Valley Ponds	1	1	1				2	5
Tralee, dam		1					1	2
'Talaheni', dam		1	1				1	3
East O'Malley, pond	1	1						2
London Bridge Rd, Burra, dam		1			2			3
Teatree Place, Jerrabomberra, dam							1	1
Head of Woolshed Creek, dam		1						1
Doyles Reserve, ephemeral wetland	1	1						2
Totals	6	12	7	2	5	2	13	